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Cobalt(II) complexes of sparfloxacin: Characterization, structure, antimicrobial activity and interaction with DNA and albumins

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ABSTRACT

The cobalt(II) complexes with the quinolone sparfloxacin (Hsf) in the absence or presence of the nitrogen-donor heterocyclic ligands 2,2′-bipyridine (bipy), 1,10-phenanthroline (phen) or 2,2′-bipyridylamine (bipyam) were prepared and characterized physicochemically and spectroscopically. The crystal structures of complexes $[Co(sf)_2(bipy)]\cdot 3MeOH\cdot 2H_2O$ and $[Co(sf)_2(phen)]\cdot 4MeOH$ were determined by X–ray crystallography. The antimicrobial activity of the complexes was tested against four different microorganisms (*Escherichia coli*, *Xanthomonas campestris*, *Staphylococcus aureus* and *Bacillus subtilis*) and was found similar or higher than that of free Hsf. The binding of the complexes to calf-thymus DNA was monitored by UV-vis spectroscopy and DNA-viscosity measurements and indirectly by competitive studies with ethidium bromide; intercalation is suggested as the most possible interaction mode. Fluorescence emission spectroscopy was used to evaluate the interaction of the complexes with human or bovine serum albumin and the corresponding binding constants were determined.

1. Introduction

Quinolones are synthetic antibacterial drugs with a 4-oxo-1,4-dihydroquinoline skeleton and are widely used for the treatment of many infections including urinary tract, respiratory and bone-joint infections, sexually transmitted diseases, prostatitis, pneumonia and acute bronchitis [1,2]. Nowadays, quinolones are among the most clinically successful antibacterial agents [3] targeting to the enzymes gyrases (type II topoisomerases) and topoisomerase IV which participate in the DNA replication [4,5]. Sparfloxacin (Hsf) (Fig. 1(A)), as a third-generation quinolone, is active against Gram-positive species such as *Staphylococci*, major respiratory pathogens and atypical pathogens responsible for pneumonia [6,7]; therefore, it is mainly used for the treatment of chronic bronchitis and community-acquired pneumonia [1]. Because of its good bioavailability and long half-life permitting one daily dose, the therapy with Hsf is more effective and of lower cost [6,7]. In regard to the metal complexes with sparfloxacin as ligand, the crystal structures of a Zn(II) [8], a Mn(II) [9], two Ni(II) [10,11] and three Cu(II) [12,13] complexes have been reported; most of the reported complexes were more active DNA-binders and potential antimicrobial agents than free Hsf [14]. Furthermore, copper(I) with sparfloxacin derivatives have been reported [15,16], as well as a series of diverse transition metal complexes of sparfloxacin [17–19] have been found in the literature.

There is only ca. 1.6 mg of cobalt in the human body but this element is crucial for coenzyme vitamin B12 (cobalamin). Recommended daily intake of vitamin B12 is 2–3 μg and its deficiency results in pernicious anemia [20]. Cobalt participates indirectly in the regulation of the DNA synthesis [21] and is also involved in cobalt-dependent proteins [21,22]. Therefore, cobalt is considered a biological element and the interest of the bioinorganic chemists has been also focused on the tentative biological activity of cobalt compounds [23]. Nowadays, doxovir (or CTC-96), a cobalt complex, has completed successfully clinical trials phase II for the treatment of herpes simplex virus [24]. A series of cobalt compounds have been reported for their antifungal [25–28], antimicrobial [29–33], antioxidant [31–37], antiparasitic [38], antipsoriatic [24], antirheumatic [39], antituberculosis [40,41], antiviral [42,43] and cytotoxic [44–47] activity and as DNA-binders [48]. In regard to structurally characterized cobalt-quinolone complexes, reports include Co(II) complexes with the quinolones ciprofloxacin [49], enoxacin [50], enrofloxacin [51], flumequine [52], oxolinic acid [29], norfloxacin and sarafloxacin [53].

We have recently initiated and reported the interaction of cobalt(II) with first- and second-generation quinolones [29,51,52]. As a continuation of this research, we present herein the synthesis, characterization and *in vitro* biological properties of cobalt(II) complexes with the

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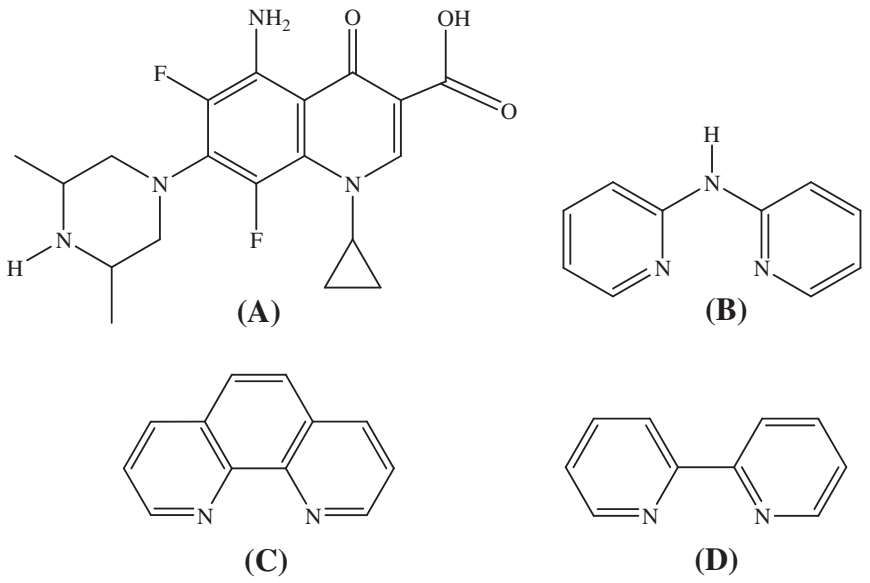

Fig. 1. The syntax formula of (A) sparfloxacin (Hsf), (B) 2,2'-bipyridylamine (bipyam), (C) 1,10-phenanthroline (phen) and (D) 2,2′-bipyridine (bipy).

third-generation quinolone sparfloxacin as ligand and the oxygen-donor methanol or the nitrogen-donors 2,2′-bipyridine (bipy), 1,10-phenanthroline (phen) and 2,2′-bipyridylamine (bipyam) (Fig. 1(B)–(D)) as co-ligands. The isolated compounds bear the formulas $[Co(sf)_2(MeOH)_2]$ for **1** and $[Co(sf)_2(L)]$, (L = bipy, phen or bipyam) for complexes **2–4,** respectively. The characterization of complexes **1–4** was achieved by physicochemical and spectroscopic techniques and X-ray crystallography for complexes $[Co(sf)_2(bipy)]\cdot 3MeOH\cdot 2H_2O$ (**2**·3MeOH·2H$_2$O) and $[Co(sf)_2(phen)]\cdot 4MeOH$ (**3**·4MeOH). The antimicrobial activity of complexes **1–4** was evaluated *in vitro* by determining the minimum inhibitory concentration (MIC) against four Gram-positive or Gram-negative microorganisms (i.e. *Escherichia coli* NCTC 29212 (*E. coli*), *Xanthomonas campestris* ATCC 1395 (*X. campestris*), *Staphylococcus aureus* ATCC 6538 (*S. aureus*) and *Bacillus subtilis* ATCC 6633 (*B. subtilis*)). The interaction mode and the binding strength of complexes **1–4** with calf-thymus (CT) DNA was investigated by UV-vis spectroscopy and viscosity measurements and by their ability to displace the well-known DNA-intercalator ethidium bromide (EB) from its CT DNA-EB conjugate examined by fluorescence emission spectroscopy. The affinity of complexes **1–4** to bind to bovine (BSA) and human (HSA) serum albumins, proteins involved in their potential transportation through the bloodstream, was investigated by fluorescence emission spectroscopy.

2. Experimental

2.1. Materials - instrumentation - physical measurements

Sparfloxacin, $CoCl_2\cdot 6H_2O$, bipy, bipyam, phen, KOH, trisodium citrate, NaCl, CT DNA, BSA, HSA and EB were purchased from Sigma-Aldrich and all solvents were purchased from Merck. All the chemicals and solvents were reagent grade and were used as purchased. DNA stock solution was prepared by dilution of CT DNA to buffer (containing 15 mM trisodium citrate and 150 mM NaCl at pH 7.0) followed by exhaustive stirring for three days, and kept at 4 °C for no longer than a week. The stock solution of CT DNA gave a ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.86, indicating that the DNA was sufficiently free of protein contamination [54]. The DNA concentration was determined by the UV absorbance at 260 nm after 1:20 dilution using $\varepsilon = 6600\ M^{-1}\ cm^{-1}$ [55].

Infrared (IR) spectra (400–4000 cm^{-1}) were recorded on a Nicolet FT-IR 6700 spectrometer with samples prepared as KBr disk. UV–visible (UV–vis) spectra were recorded as nujol mulls and in DMSO solution at concentrations in the range $10^{-5} - 5\times 10^{-3}$ M on a Hitachi U-2001 dual beam spectrophotometer. Room temperature magnetic measurements were carried out on a magnetic susceptibility balance of Sherwood Scientific (Cambridge, UK). C, H and N elemental analysis were performed on a Perkin-Elmer 240B elemental analyzer. Molar conductivity measurements of 1 mM DMSO solution of the complexes were carried out with a Crison Basic 30 conductometer. Fluorescence spectra were recorded in solution on a Hitachi F-7000 fluorescence spectrophotometer. Viscosity experiments were carried out using an ALPHA L Fungilab rotational viscometer equipped with an 18 mL LCP spindle.

2.2. Synthesis of the complexes

2.2.1. Synthesis of $[Co(sf)_2(MeOH)_2]$, *1*

A methanolic solution (10 mL) of sparfloxacin (0.5 mmol, 196 mg) and KOH (0.5 mmol, 28 mg) was stirred for 30 min. Afterwards, the solution was added drop-wise to a methanolic solution (10 mL) of $CoCl_2\cdot 6H_2O$ (0.25 mmol, 60 mg) followed by an additional 30-min stirring. The resultant solution was left for slow evaporation. Orange product of $[Co(sf)_2(MeOH)_2]$ **1** (135 mg, yield: 60%) was collected after fifteen days. Anal. calcd. for $[Co(sf)_2(MeOH)_2]$ $C_{40}H_{50}CoF_4N_8O_8$ (MW = 905.81) C, 53.04; H, 5.56; N, 12.37%; found: C, 53.23; H, 5.35; N, 12.18%. IR (KBr disk): ν_{max}, cm^{-1}; $\nu(C=O)_{pyridone}$: 1631(very strong (vs)); $\nu_{asym}(CO_2)$, 1615(vs); $\nu_{sym}(CO_2)$, 1376(strong (s)); $\Delta\nu(CO_2) = \nu_{asym}(CO_2) - \nu_{asym}(CO_2) = 239\ cm^{-1}$. UV–vis: as nujol mull, λ/nm: 652, 525, 455(shoulder (sh)), 385, 312; in DMSO, λ/nm (ε/M^{-1} cm^{-1}): 657(25), 525(80), 445(150), 383(sh) (1050), 304(6600). μ_{eff} at room temperature = 3.95 BM. Soluble in DMF and DMSO (Λ_M = 10 S cm^2 mol^{-1}, in 1 mM DMSO solution).

2.2.2. Synthesis of the complexes $[Co(sf)_2(L)]$ *2–4, (L = bipy, phen or bipyam)*

Complexes **2–4** were prepared in a similar way. More specifically, sparfloxacin (0.5 mmol, 196 mg) was dissolved in methanol (10 mL) and was deprotonated by KOH (0.5 mmol, 28 mg) after stirring for 30 min. The resultant solution was added slowly to a methanolic solution (10 mL) of $CoCl_2\cdot 6H_2O$ (0.25 mmol, 60 mg) followed by the simultaneous addition of a methanolic solution (95 mL) of bipy (0.25 mmol, 39 mg) for **2,** phen (0.25 mmol, 45 mg) for **3** or bipyam (0.25 mmol, 43 mg) for **4**. The resultant solution was stirred for 30 min and left for slow evaporation.